Hepatoprotective Activity of Boerhavia Diffusa Linn. (Nyctaginaceae) against Ibuprofen Induced Hepatotoxicity in Wistar Albino Rats

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ABSTRACT

The present study was designed to evaluate the hepatoprotective activity of different parts of Boerhavia diffusa Linn. (Nyctaginaceae) such as root and aerial parts against ibuprofen (IB) induced hepatotoxicity in Wistar albino rats. Boerhavia diffusa L. is one of the well known folklore medicinal plants. The administration of ibuprofen (500mg/kg. b. wt.) produced significant changes in the normal hepatic cells, resulting in the formation of gastric lesions, centrilobular necrosis, vacuolization, and hepatomegaly. The adverse effect of ibuprofen was reflected in the levels of biochemical parameters of liver marker enzymes such as ALT, AST, ALP, and bilirubin. The activities of natural antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and Glutathione-S-transferase (GST) were decreased significantly. The methanol extract (85%) of the root and aerial part of Boerhavia diffusa L. (500 mg/kg. b. wt.) produced remarkable changes in affected hepatic cell architecture and restored nearly normal structure and functions of hepatic cells. Similarly the different parts of the Boerhavia diffusa L. (500 mg/kg. b. wt.) restored the altered biochemical parameters of liver marker enzymes close to normal control levels. The observed results show the root of Boerhavia diffusa L. possesses more hepatoprotective efficacy than the aerial part of the same plant. The results suggest that the hydro alcoholic (15:85%) extract of Boerhavia diffusa L. possesses significant potential effect as a hepatoprotective agent.

Keywords: Boerhavia diffusa, hepatoprotective, hepatotoxicity, ibuprofen, wistar albino rats.

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INTRODUCTION

Liver is one of the most complex internal organs in the body. It plays an important role in the maintenance of the internal environment through its multiple and diverse functions. It is involved in the intermediary metabolism of proteins, fats, carbohydrates and foreign bodies and is responsible for the synthesis of a number of plasma proteins. It also plays an important role in the production of various enzymes and the formation and excretion of bile. It acts as a storehouse of proteins, glycogen, various vitamins and minerals. Hence, any injury to or impairment of its function has grave influence on the health of the affected person. Liver disease is still a worldwide health problem. Although viral infection is one of the main reasons of hepatic injury, xenobiotics, excessive drug therapy, environmental pollutants and chronic alcohol ingestion can also cause severe liver injury. Hepatotoxicity is a common undesirable finding following overdosing with non-steroidal anti-inflammatory drugs (NSAIDs) [1]. It can be present with widely varying severity and histopathological type of hepatic damages, which depend on the mode of action: direct hepatotoxicity inducing necrosis, paracetamol; biliary
toxicity inducing cholestasis, alphanaphthylisothiocyanate (ANIT), ibuprofen; steatosis, tetracyclins, hypertrophia, hyperplasia, hepatitis, etc. [2]. Ibuprofen [2-(4-isobutylphenyl) propionic acid] is a nonsteroidal antinflamatory drug (NSAID) of the 2-aryl propionic acid family [3, 4]. Ibuprofen is commonly prescribed for the treatment of fever, inflammation, and pain [5]. It is commonly known to produce hepatotoxicity, inducing cholestatic hepatitis [1]. Besides, ibuprofen was also found to be most active in impairing glucoseogenesis from lactate, and in impairing albumin synthesis in vitro [6]. Lower dose of ibuprofen also induces more histopathological lesions in rats’ liver than chlorpromazine, paracetamol and other [2]. In this context, ibuprofen was used in the present study to induce hepatotoxicity in rats as a representative toxin of the class of NSAIDs.

Ibuprofen is primarily metabolized by oxidation in the liver and the major metabolites are 2-hydroxy ibuprofen and 2-carboxy ibuprofen [7]. A wide range of possible side effects associated with the use of ibuprofen, including gastrointestinal intolerance, cardiovascular toxicity, renal toxicity and hepatotoxicity have been reported. The ibuprofen toxicity has been reviewed extensively in reference [8].

Researchers are now focusing their attention on identifying and validating plant derived substances for the treatment of various diseases [9]. Interestingly, it is estimated that more than 25% of modern medicines are directly or indirectly derived from plants. *Boerhaavia diffusa* L. (Nyctaginaceae), commonly known as ‘Punarnava’ in the Indian system of medicine, is a perennial creeper found throughout the waste lands of India. The plant which is reputed to be diuretic and laxative is given for the treatment of anasarca, ascites and jaundice [10]. The plants of *Boerhaavia diffusa* L. had been found to have diuretic, anti inflammatory, fibrinolytic, nephrotic syndrome and anti-convulsant activities [11-13]. It also reported the hepatoprotective activity of the aerial part of the plants. It is of importance to note that the inhibition of CYT P450 2E1 and antioxidant actions seem to be the common mechanism of action of herbal drugs. This plant was known to possess various medicinal properties like free radical scavenging activity, hypoglycemic and antidiabetic and hepatoprotective activity. A large number of compounds have been isolated from the roots of *B. diffusa* L.namely punarnavine, β-sitosterol, β-D glucoside tetracosanoic, hexacosanoic, stearic, palmitic, arachidonic acid, hentriacontance, ursoic acid and punarnava-voiside.

**MATERIALS AND METHODS**  
**Plant Material and Plant extract preparation**  
*Boerhavia diffusa* L. were collected from Thengal in Vellore district of Tamilnadu, India in the month of March 2010 during the early hours of the days. The plant was identified and authenticated by the department of Botany, CAHC, Melvisharam, Tamil Nadu. The root and aerial part of the plants were separated, shade dried and powdered in a micro - pulveriser and subjected to soxhlet extraction, using a hydroalcoholic (85% methanol) solvent at a temperature of 70°C. The concentrated crude extract was lyophilized into powder and was preserved in an airtight container in a deep freezer until the time of use.

**Animals**  
Thirty six albino rats were used in this study. *Wistar albino* rats weighing 175-225g in the present studies were procured from the animal house of C.Abdul Hakeem College, Melvisharam, Tamil Nadu, India. The animals were housed in a clean and well ventilated experimental unit of animal house in polypropylene cages with sterile inert husk materials as bedding. All the animals were kept under standard environmental condition at 25±2°C (12h light/12h dark cycle at room temperature) and maintained on commercial pellet diet, supplied by “HINDUSTAN LEVER” Limited Mumbai, under the trade name “Gold Mohar” Feeds, water was provided ad libitum. Experimental protocols and procedures with respect to the animals employed in this study were approved by the Animal Ethics Committee of C. Abdul Hakeem College, Melvisharam, Tamil Nadu, India. The rats were kept in animal house for ten days before starting the experiments.
In this study, Silymarin (25 mg/Kg b. wt.) was used as a standard, against ibuprofen induced acute hepatic damage in Wistar albino rats. It was undertaken to determine the hepatoprotective activities of the methanol extract of root and methanol extract of aerial part of Boerhavia diffusa Linn. in an animal model. In addition, to find the levels of liver marker enzymes, histopathological studies were done to prove their efficacy of preventive and curative role against ibuprofen toxicity in vivo.

**Experimental design**

Ibuprofen (IB) was obtained from Indian Pharmaceutical Company (IPCA) Mumbai. The animals were divided into six groups consisting of six animals each for different experiments.

**Group I** rats which served as normal control received commercial feed and distilled water,

**Group II** (intoxicated group) administrated orally by gavage, with a single dose of ibuprofen (500 mg/kg b. wt.) dissolved in water (37°C).

**Group III** was given IB with single dose (500 mg/kg b. wt.) followed by silymarine (Standard) commercial drug (25 mg/kg, b. wt.) for 30 days.

**Group IV** were intoxicated with IB (500 mg/kg b. wt.) followed by the methanol extract of the root of *Boerhavia diffusa* (500 mg/kg b.wt.) for 30 days.

**Group V** were intoxicated with IB (500 mg/kg b. wt.) followed by the methanol extract of the aerial part of *Boerhavia diffusa* (500 mg/kg b.wt.) for 30 days.

**Induction of Liver damage**

Liver damage was induced by the administration of ibuprofen orally with single dose (500mg/kg body weight) in rats. After the completion of experimental course of therapy the rats were fasted over night and blood samples were collected by cervical dislocation under light ether anesthesia. Serum samples were used for the determination of various parameters.

**Preparation of hepatic homogenate**

The liver was quickly removed, part of the liver perfused immediately with ice-cold saline (0.9% NaCl), Portion of the liver was then homogenized in chilled sodium phosphate buffer (0.1M, pH 7.4) using a Potter Elvehjehm Teflon homogenizer (Yamato L.S. G. LH-21, Japan). The homogenate thus obtained was centrifuged in a cooling centrifuge at 12,000 rpm for 30 min at 4°C to obtain a post-mitochondrial supernatant (PMS), which was used for evaluation of liver endogenous antioxidant enzymes at 4°C.

**Assessment of hepatoprotective activity**

Hepatic enzymes, AST and ALT were used as the biochemical markers of the hepatic damage and were assayed by the method as given in reference [14]. ALP activity was measured using the method as given in reference [15], and serum bilirubin was estimated by the method as given in reference [16], to assess the acute hepatic damage caused by ibuprofen.

**Antioxidant Enzyme Assay:**

The activity of superoxide dismutase (SOD) was measured by the modified method as in reference [17]. Catalase (CAT) activity was measured in liver homogenates by the method as given in reference [18]. Glutathione peroxidase (GPx) activity was assayed according to the method described as given in reference [19], and the activity of Glutathione-S-transferase was estimated by the method as given in reference [20].

**Statistical analysis**

Data were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA), followed by Scheffe post hoc test. The data were analyzed with SPSS version 16 software (SPSS Inc., Chicago, USA). Statistical significance of difference was accepted at the p-values of less than 0.05.

**RESULTS**

Ibuprofen, (NSAID) is widely used as an analgesic and antipyretic [21]. It is known to be the cause of hepatotoxicity in experimental animals and human at high dosages. It is metabolized in the liver to extractable glucuronide and sulphate conjugates. However, hepatotoxicity of ibuprofen has been attributed to the formation of toxic metabolites. Over dose of ibuprofen leads to mitochondrial dysfunction followed by acute hepatic necrosis. All these events culminate in functional and morphological changes leading to loss of integrity of cell membranes which is evidenced by the rise in levels of serum marker enzymes. This occurs because
of hepatocellular damage due to the reduced activity of the antioxidant enzymes and disturbance of Ca²⁺ homeostasis [22].

**Effect of Boerhavia diffusa on various biochemical parameters**

Experiments were carried out to obtain definite proof of hepatoprotective activity of *Boerhavia diffusa* L. in *wistar albino* rats. An experimental liver damage was induced by ibuprofen at the single dose of 500 mg/kg body weight. Phytochemical analysis showed that presence of various chemical compounds such as alkaloids, flavonoids, steroids, terpenoids, safonine, and so on [23]. Blood and liver samples were collected. Blood samples were analyzed biochemically and the levels of ALT, AST, ALP and bilirubin evaluated. A portion of the liver sample was used to study the levels of antioxidant enzymes i.e SOD, CAT, GPx and GST and another portion of the liver tissue was used to study the histopathology of hepatic cells and their protective levels.

Liver marker enzymes such as ALT, AST, ALP, and bilirubin are considered to be very sensitive and reliable for measuring hepatotoxicity as well as hepato protective effect of various compounds. Hepatic necrosis induced by ibuprofen usually associated with elevated levels of liver marker enzymes is due to the cellular leakage and loss of functional integrity of the cell membrane in liver [14].

In the present study, there was no significant difference in the serum liver marker enzymes between normal and silymarin control groups. But, a hike was seen in the levels of ALT and AST, ALP and bilirubin in ibuprofen induced group (78.18%, 81.29%, 81.02% and 95.16% (Table 1) when compared to the normal group. Toxicity of ibuprofen was reduced by *Boerhavia diffusa* L. Hence, the elevated levels of the liver marker enzymes ALT and AST, ALP and bilirubin were restored near to the normal control group by both the methanol extract of root of *Boerhavia diffusa* (36.47%, 49.72%, 39.90%, and 42.97%) and methanol extract of aerial part of *Boerhavia diffusa* (36.21%, 47.86%, 38.63%, 42.14%) respectively (Table 1).

The status of antioxidant enzymes i.e SOD, CAT, GPx and GST were measured in the hepatic tissue of various experimental groups. Among them, no difference was seen in the percentage of liver antioxidant enzymes between normal and silymarin control group animals. The administration of ibuprofen decreased the levels of the above mentioned antioxidant enzymes significantly by 65.86%, 55.18%, 40.70%, and 44.68% in this group than in normal group (Table 2). The methanol extract of root of *Boerhavia diffusa* L. elevated the decreased levels of the antioxidant enzymes i.e SOD, catalase, GPx and GST (155.90%, 114.53%, 52.04%, and 69.07%) and the methanol extract of aerial part of *Boerhavia diffusa* also elevated the decreased levels of SOD, CAT, GPx, and GST (143.30%, 112.07%, 44.60%, and 55.61%) respectively (Table 2). Histopathological findings (Figure 1) (Fig. A): Shows normal hepatic cell architecture. (Fig. B) Ibuprofen intoxicated group: Shows abnormal cell architecture and centrilobular necrosis. (Fig. C) Silymarin treated liver shows nearly normal cell architecture. (Fig. D) *Boerhavia diffusa* root extract restored normal cell architecture. (Fig. F) *Boerhavia diffusa* aerial part treated liver shows reappeared cells and brought nearly normal cell architecture.

**DISCUSSION**

The present studies revealed that the roots of *Boerhavia diffusa* L. possessed marked hepatoprotective activity against ibuprofen induced hepatotoxicity than the aerial part of the plant. Due to the absence of reliable hepatoprotective drugs in the allopathic medicines, many of the natural plant products were used in therapy of different diseases [24, 25]. Different parts of the plant were used in the treatment of cancer, jaundice, dyspepsia, inflammation, spleenomegale, abdominal pain and as an anti-stress agent [13]. Many of the tribal people in India have been using the root of the *Boerhavia diffusa* L. in the treatment of liver disorders [26].

In this study, ibuprofen was used as a tool to catalyze the hepatotoxicity in *Wistar albino* rat. Various theories have been proposed for the mechanism by which ibuprofen damages the liver [27]. It was reported to cause changes in the architecture of the hepatic cell, cell permeability and to create ionic imbalance resulting in increased intracellular calcium concentration. Consequently, mitochondrial activity was...
inhibited, leading to the death of hepatic cells [28]. Ibuprofen has been used as a drug to induce the hepatotoxicity in the experimental animals. This drug caused per oxidative degeneration in the adipose tissue resulting in the fatty change and infiltration of the hepatocytes. Hike of the liver marker enzymes exhibit the cellular leakage and loss of functional integrity of the cell membrane in liver [14]. The administration of 500 mg/Kg b. wt. of methanol root and aerial part (separately) extract of Boerhavia diffusa revealed significant reduction in the elevated levels of liver marker enzymes. This shows the hepatoprotective effect of Boerhavia diffusa. It also shows that the hike of antioxidant enzymes i.e SOD, CAT, GPx and GST in the ibuprofen administrated groups increased after the administration of the plant extract. These results proved the potential hepato protective effect of the root and aerial part (separate) of Boerhavia diffusa. The root of the plant possesses more hepatoprotective efficacy than the aerial parts. In addition, these extracts are efficient in the activity of scavenging free radicals. The above mentioned extracts brought down IB induced damage in cells to the normal cell architecture in the liver of ibuprofen intoxicated rats.

Table 1: Activity levels of serum Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline Phosphate (ALP), and Bilirubin in normal, Ibuprofen intoxicated and plant extracts treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters in the serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT (IU/l/min/mg protein)</td>
</tr>
<tr>
<td>Group-I (NaCl-normal control)</td>
<td>58.23±1.15</td>
</tr>
<tr>
<td>Group-II (IB+Silymarin control)</td>
<td>60.14±1.33b</td>
</tr>
<tr>
<td>% of change (Normal vs Silymarin)</td>
<td>-42.04</td>
</tr>
<tr>
<td>Group-III IB Control (500mg/kg)</td>
<td>103.76±1.05a</td>
</tr>
<tr>
<td>% of change (Normal vs IB)</td>
<td>+78.18</td>
</tr>
<tr>
<td>Group-IV IB+Bd (root-500mg/kg)</td>
<td>65.91±1.58b</td>
</tr>
<tr>
<td>% of Changes IB vs Bd (root)</td>
<td>-36.47</td>
</tr>
<tr>
<td>Group-V IB+Bd (aerial part 500mg/kg)</td>
<td>66.18±1.49b</td>
</tr>
<tr>
<td>% of Changes IB vs Bd (aerial part)</td>
<td>-36.21</td>
</tr>
</tbody>
</table>

Values are mean ± S.D., n = 6. ‘a’; p < 0.05 compared with normal control. ‘b’ p < 0.05 compared with IB intoxicated. + or – indicates the changes of percentage.
Table 2: Levels of SOD, CAT, GPX and GST in the liver homogenate of the normal, Ibuprofen intoxicated and plant extracts treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters in the liver (mg/dl)</th>
<th>Groups</th>
<th>Parameters in the liver (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOD (mg/dl)</td>
<td>CAT (mg/dl)</td>
<td>GPX (mg/dl)</td>
</tr>
<tr>
<td>Group-I (NaCl-normal control)</td>
<td>3.72±1.15</td>
<td>92.24±1.18</td>
<td>11.57±1.69</td>
</tr>
<tr>
<td>Group-II (IB+Silymarin control)</td>
<td>3.51±1.31^b</td>
<td>90.15±1.71^b</td>
<td>11.19±1.28^b</td>
</tr>
<tr>
<td></td>
<td>% of change (Silymarin vs Normal)</td>
<td>+176.37</td>
<td>+118.06</td>
</tr>
<tr>
<td>Group-III IB Control (500mg/kg)</td>
<td>1.27±1.15^a</td>
<td>41.34±1.72^a</td>
<td>6.86±1.36^a</td>
</tr>
<tr>
<td></td>
<td>% of change (IB vs Normal)</td>
<td>-65.86</td>
<td>-55.18</td>
</tr>
<tr>
<td>Group -IV IB + Bd (root-500mg/kg)</td>
<td>3.25±1.72^b</td>
<td>88.69±1.32^b</td>
<td>10.43±1.96^b</td>
</tr>
<tr>
<td></td>
<td>% of Changes IB vs Bd (root)</td>
<td>+155.90</td>
<td>+114.53</td>
</tr>
<tr>
<td>Group -V IB + Bd (aerial part-500mg/kg)</td>
<td>3.09±2.25^b</td>
<td>87.67±1.52^b</td>
<td>9.92±1.64^b</td>
</tr>
<tr>
<td></td>
<td>% of Changes IB vs Bd (aerial part)</td>
<td>+143.30</td>
<td>+112.07</td>
</tr>
</tbody>
</table>

Values are mean ± S.D., n = 6. 'a' - p < 0.05 compare with normal control. 'b' - p < 0.05 compare with IB intoxicated. 'P' denotes statistical significance.

Figure: 1 Histopathological plates

Fig. A: Control: Shows Normal architecture

Fig. B: Ibuprofen intoxicated cell: Shows abnormal cell architecture, centrilobular necrosis

Fig. C: Ibuprofen + Sylimarin treated: Shows nearly normal cell architecture

Fig. D: Ibuprofen (500mg/kg b.wt.) intoxicated + Boerhavia diffusa root (500 mg/kg b. wt.): Shows nearly Normal cell architecture
CONCLUSION
The experimental results suggest that the plant *Boerhavia diffusa* is a hepatoprotectant. The different active components are found in *Bd*, which may be responsible for the actual hepatoprotectivity. The present study suggested that the *Boerhavia diffusa* has a preventive and curative effect in Ibuprofen induced hepatotoxicity in albino rats. From the above study, we can conclude that this plant has medicinal properties. However, further investigations and analysis are required in order to establish the active compounds which are responsible for the hepatoprotective properties.

REFERENCES